Europ.J.Hort.Sci., **69** (3). S. 117–124, 2004, ISSN 1611-4426. © Verlag Eugen Ulmer GmbH & Co. Stuttgart

# Qualitative and Quantitative Composition of the Flavour Components of Taiwan Calamondin and Philippine Calamansi Fruit

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## Summary

A solid-phase micro-extraction (SPME) procedure for analysis of the flavour components of two varieties of *Citrus madurensis* Lour. (Syn. *Citrus microcarpa* Bunge), the Philippine calamansi and the Taiwan calamondin, is presented. Fifty-eight components were identified, and grouped into eight classes: 8 monoterpene hydrocarbons, 3 oxygenated monoterpenes, 18 sesquiterpenes, 2 aliphatic hydrocarbons, 10 esters, 11 aldehydes, 3 alcohols and 3 ketones. On the whole, Philippine calamansi juice has statistically higher percentage of ketones, monoterpene hydrocarbons, and sesquiterpenes than calamondin from Taiwan. Principal component analysis (PCA) was used as a descriptive tool to identify aroma species that contributed strongly to overall flavour constituent's variation. Five principal components were extracted from the data matrix represent a cumulative 84.1 % of the total variation of aroma concentration. Among the five PCA axes, the distribution of the Philippine calamansi samples was separated nicely from the distribution of the Taiwan calamondin samples along the third and fourth axes graphically. The third axis is the axis of ß-pinene and ß-cubebene. While nonyl alcohol and linalyl alcohol correlate greatly to the fourth axis. The key components occurring in the Taiwan calamondin and Philippine calamansi were compared and discussed.

## Zusammenfassung

**Qualitative und quantitative Zusammensetzung der Geschmackskomponenten von Calamondin und Calamansi.** Es wird eine Festphasen-Mikroextraktion-Methode (SPME) zur Analyse der Geschmackskomponenten von zwei Varietäten von *Citrus madurensis* Lour. (Syn. *Citrus microcarpa* Bunge), der philippinischen Calamansi und der taiwanesischen Calamondin vorgestellt. 58 Komponenten wurden identifiziert und in acht Gruppen eingeteilt: 8 Monoterpenkarbone und 3 oxygenierte Monoterpene, 18 Sesquiterpene, 2 aliphatische Hydrokarbone, 10 Ester, 11 Aldehyde, 3 Alkohole und 3 Ketone. Im großen und ganzen hatte Calamansi-Saft statistisch abgesichert höhere Prozentanteile an Ketonen, Monoterpenkarbonen und Sesquiterpenen als der Calamondin-Saft. Die Hauptkomponenten-Analyse (PCA) wurde als beschreibendes Werkzeug zur Identifizierung der Aromakomponenten, die hauptsächlich zur Variation der Gesamtgeschmackbestandteile beitrugen, verwendet. Fünf Hauptkomponenten wurden aus dem Datenmaterial zusammengestellt, die 84.1 % der Gesamtvariation der Aromen repräsentieren. Innerhalb der fünf PCA-Achsen trennten sich die Calamansi-Proben entlang der dritten und vierten Achse deutlich von den Calamondin-Proben. Die dritte Achse ist die Achse der β-Pinene und β-Kubebene. Die vierte Achse korreliert stark mit Nonyl- und Linalylalkohol. Die Schlüsselkomponenten, die in der philippinischen Calamansi und der taiwanesischen Calamondin auftreten, wurden verglichen und diskutiert.

**Key words.** calamansi – calamondin – citrus fruits – fragrance – PCA – SPME – terpenes – aliphatic alcohols – ß-pinene – ß-cubebene.

## Introduction

*Citrus madurensis* Lour. (syn. *Citrus microcarpa* Bunge) is widely distributed in southeast Asia. There are many common names for this species, including calamondin, kalamondin, kalamunding, kalamansi, calamansi, limmonsito and agridulce, with calamansi being the most common in the Philippines, and calamondin in Taiwan.

Calamondin or calamansi fruit has an average diameter of 3.3 cm. Rind glossy green color turns orange to orange-red; thin, smooth and pitted, easily separable at maturity. The fruit is used primarily for its juice as hot drink due to its low level of sugars (less than 1.5 %), rich volatile components, ascorbic acid, and citric acid (NISPEROS-CARRIEDO 1992).

Both the Philippine calamansi and Taiwan calamondin originate from southern China (LI et al. 1979; MORTON 1987). The calamondin was introduced to Taiwan in 1694 (MORTON 1987). The Philippine calamansi was introduced to Taiwan in the early 1990's (LIAO et al. 1998). The introduced calamansi plants were in full production by 1998.

The Taiwan calamondin and the Philippine calamansi essentially belong to the same species. However, when comparing the morphological characteristics between calamondin acclimated and long-cultivated in

Taiwan and the calamansi introduced from the Philippines, there are many differences (LIAO et al. 1998). Furthermore there is a distinct difference in aroma when served as a hot drink. Therefore, the main purpose of this study is to differentiate the key flavour components in terms of instrumentation.

## Materials and Methods

*Sample collection*

Philippine calamansi (*Citrus madurensis* Lour; from Philippine) fruit were picked from a 6-year-old seed-grown tree planted at the Chia-yi Agricultural Experimental Station, Chia-yi, Taiwan (23 °N latitude). Taiwanese calamondin (*Citrus madurensis* Lour; from Taiwan) fruits were collected from a five-year-old tree, where the calamondin scion was grafted on to a Canton lemon (*Citrus limonia* Osbeck; Rangpur lime) as stock (LI et al. 1979). Both the Taiwan calamondin and Philippine calamansi trees were mature and in full fruit production. Ten fruits from each variety were collected for this study.

*Sample preparation*

Calamansi and calamondin fruits are small compared to other citrus fruits (about 3.3 cm in diameter); therefore, the juice could be extracted from the fruit by squeezing them manually. The squeezed fruit samples were centrifuged to remove the debris at a speed of 3000 rpm to provide pure juice samples for this study.

*Materials*

A solid-phase micro-extraction (SPME) (SCHEPPER WERCINSKI 1999) system, including reaction vials, extraction stand and fibre tower, was purchased from Supelco (Bullebonte, PA, USA). We selected a Supelco 65 μm carbowax/divinylbenzene coating fibre for this study since it has a strong affinity for a wide spectrum of juice volatile and nonvolatile flavour components including hydrocarbons, alcohols, esters, aldehydes and ketones. A Hewlett Packard (Palo Alto, CA, USA) gas chromatograph/mass spectrometer (GC/MS) 5973-system was used for identifying and quantifying each chemical species. A Restak (Bellebonte, PA, USA) Stablewax DA (30 m x 0.25 mm, 0.25 um column thickness) fused-silica capillary column was used for compound separation in this study.

*Sample Analysis*

To prepare the samples, 1.00 g of juice was given into a 4 ml extraction vial, and 2 ml de-ionized water was added. To eliminate the random error due to the extraction and analysis procedures, we spiked the sample with 100 ng of phenol D6 as an internal standard. The juice sample was maintained at 25±1 °C and stirred at 100 rpm. The fibre needle was inserted into the vial and the adsorption fibre was allowed to soak in the juice sample for 20 minutes. The absorption fibre was retrieved and introduced into the GC injection port. The injection port temperature was set at 250 °C. The fibre was heated in the injection port for 3 minutes for thermal desorption prior to release into the column. The column temperature was initially held at 100 °C for 2 minutes then increased at 8 °C/min to 250 °C and finally held at 250 °C for 9.25 minutes. The detector was set at 250 °C. The mass range was scanned from m/z 30 to 500 every 2 seconds. The electron impact was set at 70 eV. A Hewlett Packard Chem Station was used for data processing. The mass spectrum from each chromatographic peak was identified, by searching and comparing the mass spectrum with the Hewlett Packard NBS75K mass spectrum library. The total ion chromatogram (TIC) of each peak was integrated. The quantity of the compound was calculated by using the response factor of phenol D6. The extraction fibre was sterilized at 250 °C for one hour prior to the first batch run of the day. A blank and a spiked blank check were run daily to ensure the quality of the analysis. 10 replicates of each sample were analysed.

*Data Analysis*

One-way analysis of variance (ANOVA) was used for statistical analysis of the compound differences between the two varieties. In order to describe the aroma patterns of calamansi and calamondin, principal component analysis (PCA) was used as a tool to reduce the set of correlated variables to a smaller set of statistically independent components. PCA was performed on covariance matrices derived from pairwise analyses of natural-log-transformed variables. A statistical software package STATISTICA was used to perform the analysis.

## Results

The total ion chromatogram (TIC) profiles of the aroma constituents from the fruit juices of the Philippine calamansi and Taiwan calamondin are shown in Fig. 1. Fifty-eight components were identified, and grouped into eight classes: 8 monoterpene hydrocarbons, 3 oxygenated monoterpenes, 18 sesquiterpenes, 2 aliphatic hydrocarbons, 10 esters, 11 aldehydes, 3 alcohols and 3 ketones. The concentration of each compound is listed in Table 1, for comparison of the main aroma constituents.

We used principal component analysis (PCA) as a descriptive tool to identify aroma species that contributed strongly to overall aroma constituent's variation. We also used a scree test to determine how many factors should be retained. Table 2 presents the factor loadings and percentages of variance explained for principal components from analyses based on the covariance matrix of log-transformed variables for aroma concentration of the fruit juices of the Philippine calamansi and Taiwan calamondin. Five principal components were extracted from the data matrix representing a cumulative 84.1 % of the total variation of aroma concentration. Principal component 1 represents 34.4 % of the total variation and is essentially a factor of terpenes with high positive loadings for monoterpenes and sesquiterpenes. The 2nd principal component represents 19.6 % of the variation, with high positive loading for (-)-β-elemene and negative loading for octyl alcohol, elixene and eudesmene. Principal component 3 represents

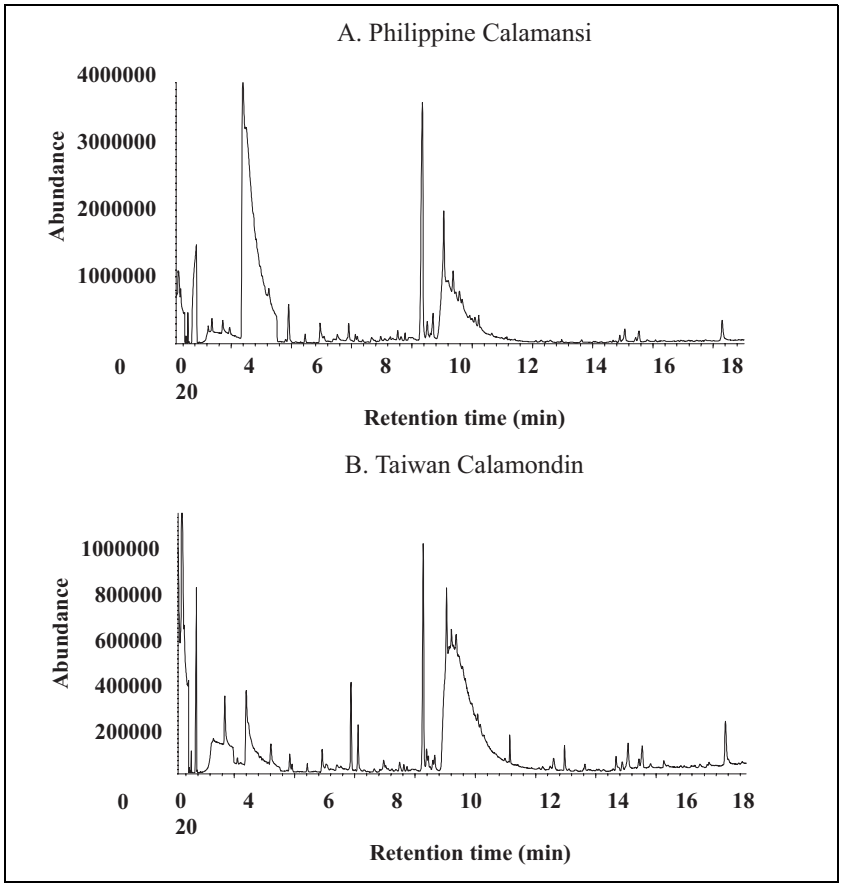



Fig. 1. Representative total ion chromatograms of fruit juice from Philippine Calamasi and Taiwan Calamondin.

12.8 % of the variation with high positive loadings for ß-pinene and ß-cubebene and high negative loading for (-)-aristolene. Principal component 4 representing 9.2 % of the variation is a factor of aliphatic alcohol with high positive loadings for linalyl alcohol and high negative loadings for nonyl alcohol. Principal component 5 represents 8.1 % of the variation with high positive loading for calarene and high negative loading for β-eudesmol.

Among the five principal components, we found the distribution of the Philippine calamansi samples was separated graphically from the distribution of the Taiwan calamondin samples along the 3rd and 4th axes. Therefore, we plotted the individual samples of the Philippine calamansi and Taiwan calamondin in multivariate aroma component space defined by the 3rd and 4th axes derived from the principal component analysis. Fig. 2 shows that the distribution of the Philippine calamansi samples was separated nicely from the distribution of the Taiwan calamondin samples along the axes of aliphatic alcohols and the axis of the combination of ß-pinene and ß-cubebene.

In order to compare the aroma profiles of the fruit juices, we calculated the percentage aroma component of each sample based on 25 compounds which were detected at least at 50 % of frequency above the detection limits (1 ng $g^{-1}$ of fruit juice) for either samples of Philippine calamansi or Taiwan calamondin. Both fruit juices share similar aliphatic alcohols, esters and oxgenated monoterpene profiles (Table 3). However, the percentage of ketone, monoterpene hydrocarbon and sesquiterpene constituents make the aroma profiles of the two fruit juices different. On the whole, Philippine calamansi juice has statistically higher percentages of ketones, monoterpene hydrocarbons, and sesquiterpenes than calamondin from Taiwan.

Both fruit juices share similar alcohol profiles. However, Philippine calamansi juice has a higher 1-octanol concentration than calamondin from Taiwan (Table 1).

There is no significant difference in the quantity of aldehyde compounds in both juices except for decanal. The calamansi juice has a greater quantity of decanal than the calamondin (Table 2).

The ester profiles between the two fruit juices are dramatically different (Table 1). This may contribute to the aroma-taste difference between the two cultivars. However, the quantitative differences of flavour on the two cultivars corresponding with different compounds need to be evaluated and tested.

Terpene hydrocarbons are the key components of citrus essential oil. They are important aroma ingredients in citrus fruits. Both types of fruit juice shared the same monoterpene and sesquiterpene profiles. However, the monoterpene hydrocarbons including ß-pinene, ß-myrcene, ß-phellandrene and limonene as well as sesquiterpene including ß-cubebene, calarene, eudesmene, elixene, muurolene, elemol, (+)-cadinene and β-eudesmol found in the juice of the Philippine calamansi are higher than those found in the juice of the Taiwan calamondin (Table 1).

Table 1. A comparison of the tentatively identified volatile and non volatile fragrance compounds in fruit of the Taiwan calamondin and Philippine calamansi.

| TIC | Eluting sequence | Retaining time index | IUPAC or synonym | Taiwan calamondin | | Philippine calamansi | | |
|---|---|---|---|---|---|---|---|---|
| | | | | Mean* | N | Mean* | N | F-test |
| **Monoterpene hydrocarbons** | | | | | | | | |
| β-pinene | 1 | 0.172 | Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene- | *7 | 10 | 56 | 10 | P<0.01 |
| β-myrcene | 2 | 0.176 | 1,6-Octadiene, 7-methyl-3-methylene- | 35 | 10 | 140 | 10 | P<0.01 |
| limonene2 | 3 | 0.194 | Cyclohexene, 1-methyl-4-(1-methylethenyl)- | 5083 | 10 | 9168 | 10 | ns |
| β-phellandrene | 4 | 0.203 | Cyclohexene, 3-methylene-6-(1-methylethyl)- | 66 | 5 | 644 | 3 | P<0.01 |
| 3-carene | 5 | 0.215 | Bicyclo[4.1.0]hept-3-ene, 3,7,7-trimethyl- | 72 | 3 | | | NA |
| limonene1 | 9 | 0.3 | Cyclohexene, 1-methyl-4-(1-methylethenyl)-, R) | 14149 | 10 | 50214 | 10 | P<0.01 |
| m-mentha-1,8-diene | 36 | 0.709 | Cyclohexene, 1-methyl-3-(1-methyl- ethenyl)-, (.+-.)- | | | 404 | 1 | NA |
| terpinolene | 40 | 0.717 | Cyclohexene, 1-methyl-4-(1-methylethylidene)- | 68 | 3 | | | NA |
| **Oxygenated monoterpenes** | | | | | | | | |
| linalyl alcohol | 16 | 0.539 | 1,6-Octadien-3-ol, 3,7-dimethyl- | 336 | 9 | 569 | 9 | ns |
| 4-terpineol | 24 | 0.614 | 3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)- | 94 | 8 | 161 | 8 | ns |
| terpieol | 37 | 0.711 | 3-Cyclohexene-1-methanol, alpha,.alpha.,4-trimethyl-, (S)- | 124 | 1 | 64 | 1 | NA |
| **Sesquiterpenes** | | | | | | | | |
| δ-elemene | 12 | 0.483 | Cyclohexene, 4-ethenyl-4-methyl-3-(1-methyl-ethenyl)-1-(1-methylethyl)-, (3R-trans)- | 252 | 8 | 344 | 9 | ns |
| β-caryophyllene | 19 | 0.574 | Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimeth-yl-8-methylene-, [1R-(1R*,4E,9S*)]- | | | 90 | 1 | NA |
| (-)-β-elemene | 21 | 0.588 | Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-, [1S-(1.alpha.,2.beta.,4.beta.)]- | 71 | 2 | 90 | 4 | ns |
| β-farnesene | 25 | 0.632 | 1,6,10-Dodecatriene, 7,11-dimethyl-3-methyl-ene-, (E)- | | | 112 | 2 | NA |
| germacrene D | 27 | 0.642 | 1,6-Cyclodecadiene, 1-methyl-5-me- thyl-ene-8-(1-methylethyl)-, [s-(E,E)]- | | | 131 | 1 | NA |
| β-cubebene | 30 | 0.668 | 1H-Cyclopenta[1,3]cyclopropa[1,2]benzene, octahydro-7-methyl-3- methylene | 121 | 4 | 3607 | 9 | P<0.01 |
| germacrene B | 31 | 0.676 | 1,5-Cyclodecadiene, 1,5-dimethyl-8- (1-meth-ylethylidene)-, (E,E)- | | | 132 | 1 | NA |
| calarene | 32 | 0.678 | 1H-Cyclopropa[a]naphthalene, 1a,2,3,5,6,7,7a,7b-octahydro-1,1,7,7a-tetrame-thyl-,[1aR-(1a.alpha. ,7.alpha., 7a.alpha.,7b.al-pha.)]- | 638 | 6 | 181 | 2 | P<0.01 |
| δ-cadinene | 33 | 0.686 | Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dime-thyl-1-(1-methylethyl)-, (1S-cis)- | | | 194 | 1 | NA |
| eudesmene | 35 | 0.705 | Naphthalene, decahydro -4a-methyl -1-meth-ylene-7-(1-methylethenyl)-, [4aR-(4a.al-pha.,7.alpha.,8a.beta.)]- | 411 | 4 | 161 | 4 | P<0.05 |
| α-guaiene | 38 | 0.711 | 3-Cyclohexene-1-methanol, .alpha.,.al-pha.,4-trimethyl-, (S)- | | | 280 | 1 | NA |
| elixene | 41 | 0.718 | Cyclohexane, 1-ethenyl-1-methyl-2-(1-methyl-ethenyl)-4-(1-methylethylidene)- | 108 | 4 | 2576 | 6 | P<0.01 |
| muurolene | 42 | 0.728 | Naphthalene, 1,2,3,4,4a,5,6,8a-octahy-dro-7-methyl-4-methylene-1-(1-methyl | 502 | 7 | 1143 | 9 | P<0.05 |
| aromadendrene, (+)- | 44 | 0.738 | 1H-Cycloprop[e]azulene, decahydro-1,1,7-trim-ethyl-4-methylene-,[1aR-(1a.alpha.,4a.alpha.,7.al-pha.,7a.beta.,7b.alpha.)]- | 171 | 1 | 394 | 2 | NA |
| elemol | 49 | 1.071 | Cyclohexanemethanol, 4-ethenyl-.alpha.,.al-pha.,4-trimethyl-3-(1-methylethe-nyl)-,[1R-(1.alpha.,3.alpha.,4.beta.)] | 88 | 6 | 138 | 10 | P<0.01 |
| (-)-aristolene | 51 | 1.165 | (-)-Aristolene | 55 | 6 | 85 | 1 | NA |
| (+-)-cadinene | 52 | 1.195 | Naphthalene, 1,2,4a,5,8,8a-hexahy-dro-4,7-dimethyl-1-(1-methylethyl)-,(1.al-pha.,4a.beta.,8a.alpha.)-(.+/-.)- | 71 | 10 | 148 | 10 | P<0.01 |

| TIC | Eluting sequence | Retaining time index | IUPAC or synonym | Taiwan calamondin | | Philippine calamansi | | |
|---|---|---|---|---|---|---|---|---|
| | | | | Mean* | N | Mean* | N | F-test |
| **Sesquiterpenes** | | | | | | | | |
| β-eudesmol | 53 | 1.202 | 2-Naphthalenemethanol, decahydro-.alpha.,.alpha.,4a-trimethyl-8-methylene | 227 | 8 | 366 | 10 | P<0.05 |
| **Aliphatic hydrocarbons** | | | | | | | | |
| docosane | 55 | 1.29 | n-docosane | 63 | 1 | 55 | 1 | NA |
| docosane, 9-butyl- | 56 | 1.353 | 9-n-butyldocosane | 111 | 3 | | | NA |
| **Ester** | | | | | | | | |
| acetic acid cis-3-hexenyl ester | 7 | 0.266 | 3-Hexen-1-ol, acetate, (Z)- | 214 | 7 | | | NA |
| 4-penten-1-ol, 3-methyl-, acetate | 8 | 0.281 | 3-Methyl-4-penten-1-ol acetate | 26 | 1 | | | NA |
| octyl acetate | 11 | 0.448 | Acetic acid, octyl ester | | | 121 | 8 | NA |
| octyl formate | 18 | 0.557 | Formic acid, octyl ester | | | 212 | 1 | NA |
| decyl acetate | 29 | 0.664 | n-decyl acetate | | | 30 | 9 | NA |
| linalyl propanoate | 39 | 0.716 | linalyl propanoate | 93 | 1 | | | NA |
| carvyl acetate | 43 | 0.735 | 2-Cyclohexen-1-ol, 2-methyl-5- (1-methylethenyl)-, acetate, cis- | 65 | 1 | | | NA |
| geraniol acetate | 45 | 0.74 | 2,6-Octadien-1-ol, 3,7-dimethyl-, acetate, (E)- | 313 | 7 | | | NA |
| phenylethyl benzoate | 50 | 1.114 | Benzoic acid, 2-phenylethyl ester | | | 52 | 1 | NA |
| dibutyl phthalate | 58 | 1.55 | 1,2-Benzenedicarboxylic acid, dibutyl ester | | | 99 | 5 | NA |
| **Aldehyde** | | | | | | | | |
| octanal | 6 | 0.238 | n-caprylaldehyde | 132 | 3 | 335 | 9 | ns |
| nonanal | 10 | 0.384 | n-nonaldehyde | 133 | 10 | 139 | 1 | NA |
| decanal | 13 | 0.486 | n-decaldehyde | 101 | 6 | 353 | 6 | P<0.05 |
| 2-furaldehyde | 14 | 0.489 | 2-Furancarboxaldehyde | 157 | 10 | 245 | 5 | ns |
| 3-furaldehyde | 15 | 0.502 | 3-Furancarboxaldehyde | | | 106 | 3 | NA |
| undecanal | 20 | 0.584 | n-undecanal | | | 62 | 2 | NA |
| tridecanal | 22 | 0.594 | n-tridecylaldehyde | | | 138 | 1 | NA |
| 5-methylfurfural | 23 | 0.611 | 2-Furancarboxaldehyde, 5-methyl- | 98 | 10 | 146 | 10 | ns |
| 2-decenal, (E)- | 28 | 0.664 | Acetic acid, decyl ester | 82 | 2 | 245 | 2 | NA |
| β-citral | 34 | 0.693 | 2,6-Octadienal, 3,7-dimethyl-, (Z)- | 48 | 2 | | | NA |
| hydroxymethylfurfurol | 57 | 1.39 | 2-Furancarboxaldehyde, 5-(hydroxymethyl)- | 549 | 10 | 673 | 10 | ns |
| **Alcohols** | | | | | | | | |
| 1-octanol | 17 | 0.554 | 1-Octanol | 176 | 10 | 551 | 9 | P<0.01 |
| nonyl alcohol | 26 | 0.639 | Nonanol | 84 | 3 | 106 | 3 | ns |
| 6-methyl-2-pyrazinyl-methanol | 48 | 0.993 | 6-Methyl-2-pyrazinylmethanol | | | 71 | 2 | NA |
| **Ketone** | | | | | | | | |
| methylmaleic anhydride | 46 | 0.76 | 2,5-Furandione, 3-methyl- | 17395 | 10 | 23282 | 10 | ns |
| furaltadone | 47 | 0.97 | 2-Oxazolidinone, 5-(4-morpholinylmethyl)-3-[[(5-nitro-2-furanyl)methylene]amino]- | 83 | 2 | | | NA |
| 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- | 54 | 1.221 | 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- | 58 | 5 | 91 | 5 | ns |

*: ng/g of fruit juice, N: frequency detected out of 10 samples, NA: Not enough detected values to perform statistical analysis.
F-test: ns stands for no significance; p<0.05 indicates significantly different at 95 % level, while p<0.01 indicates significantly difference at 99 % level.
Eluting sequence: The number represents the eluting order of each peak by using a Stablewax DA (30 m x 0.25 mm, 0.25 um column thickness) fused-silica capillary column. The GC oven was initially held at 100 °C for 2 minutes then increased at 8 °C/min to 250 °C and finally held at 250 °C for 9.25 minutes.
Retaining time index: Mean relative retention time compared to the retention time of Phenol D6 (mean retention time is 14.610 minutes).
TIC: Tentative identified compounds

Table 2. Factor loadings for the first five principal components. Bolded faced indices indicate the significant ($p<0.05$ or better) loadings.

| Tentative identified compounds | Factor 1 | Factor 2 | Factor 3 | Factor 4 | Factor 5 |
|---|---|---|---|---|---|
| **Aliphatic alcohols** | | | | | |
| nonyl alcohol | -0.2081 | 0.0788 | 0.1337 | **-0.9039** | 0.0973 |
| octyl alcohol | 0.3064 | **-0.7544** | -0.0968 | -0.2358 | -0.3074 |
| **Oxygenated monoterpenes** | | | | | |
| linalyl alcohol | -0.1673 | -0.1446 | 0.1974 | **0.8511** | 0.0253 |
| **Monoterpene hydrocarbons** | | | | | |
| β-phellandrene | **0.8181** | -0.3654 | -0.2208 | -0.0653 | -0.0054 |
| β-pinene | **0.5340** | -0.1099 | **0.7548** | 0.1030 | -0.0858 |
| limonene 1 | **0.8868** | -0.0235 | 0.2782 | 0.0947 | -0.1378 |
| limonene 2 | **0.9421** | -0.0345 | 0.0061 | 0.0329 | -0.1202 |
| **Sesquiterpenes** | | | | | |
| (-)-aristolene | 0.2567 | 0.2130 | **-0.6870** | -0.1106 | 0.3958 |
| muurolene | **0.8212** | 0.1925 | -0.2303 | -0.0489 | -0.3519 |
| calarene | -0.1817 | 0.1598 | -0.0820 | -0.0409 | **0.8818** |
| β-cubebene | -0.0346 | 0.1212 | **0.9123** | -0.0931 | 0.0666 |
| (.+-.)-cadinene | **0.7284** | -0.3844 | 0.3668 | 0.0793 | 0.2810 |
| (-)-β-elemene | 0.2880 | **0.7634** | 0.1972 | -0.3019 | 0.0853 |
| δ-elemene | **0.8042** | 0.2547 | 0.0562 | -0.1597 | 0.0772 |
| elixene | 0.3051 | **-0.6795** | 0.4398 | 0.2854 | -0.1014 |
| eudesmene | -0.1840 | **-0.7394** | 0.1921 | 0.1990 | 0.2032 |
| β-eudesmol | **0.7187** | 0.1675 | 0.1082 | 0.1895 | **-0.5572** |
| **Eigenvalue** | 5.853 | 3.329 | 2.174 | 1.568 | 1.379 |
| **% variance** | 34.4 | 19.6 | 12.8 | 9.2 | 8.1 |

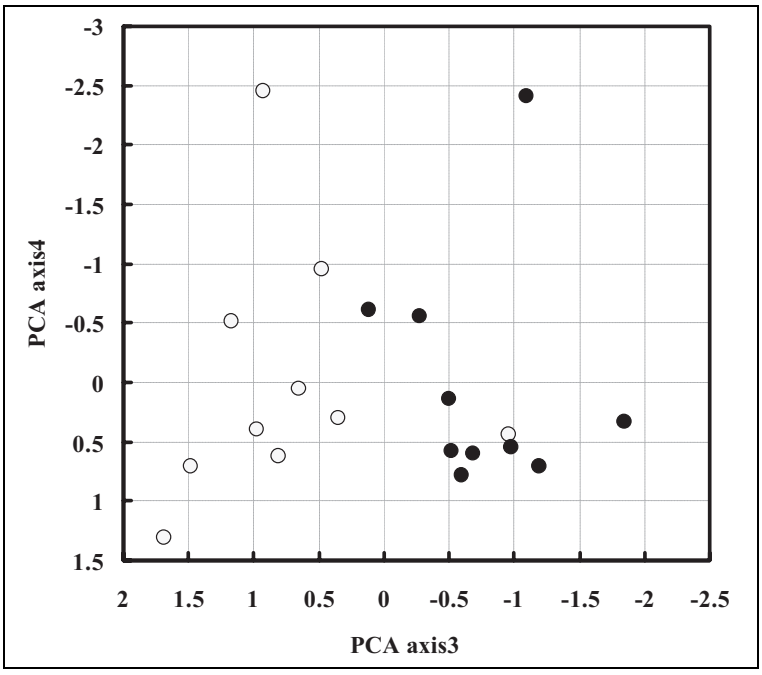



Fig. 2. Distribution of samples for Philippian calamansi (o) and Taiwan calamondin (•) along the third and forth principal component axis; β-pinene and β-cubebene vs. aliphatic alcohols.

The mean percentage of total ketones found in the juice of the Philippine calamansi is higher than that found in the juice of the Taiwan calamondin. However, there is no significant difference on the concentration of each individual ketone constituent between the two cultivars (Table 1).

## Discussion

The Philippine calamansi and the Taiwan calamondin grow in different geographical regions and have acclimated to their own ecological niches. Although they are botanically the same species (*Citrus madurensis*

Table 3. A comparison of the percentage of volatile and non volatile fragrance substances in the fruit of the Taiwan calamondin and Philippine calamansi.

| Tentative identified compounds | Taiwan calamondin * | Philippine calamansi * | F-test |
|---|---|---|---|
| **Alcohols** | 0.80 | *0.95 | ns |
| nonyl alcohol | 0.24 | 0.32 | ns |
| octyl alcohol | 0.56 | 0.63 | ns |
| **Esters** | **0.42** | **0.45** | **ns** |
| decyl acetate | t | 0.30 | NA |
| octyl acetate | t | 0.15 | NA |
| acetic acid cis-3-hexenyl ester | 0.42 | t | NA |
| **Ketones** | **28.25** | **37.36** | **p<0.01** |
| 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- | 0.24 | 0.23 | ns |
| methylmaleic anhydride | 28.02 | 37.14 | ns |
| **Monoterpene hydrocarbons** | **36.31** | **50.74** | **p<0.05** |
| β-phellandrene | 0.22 | 0.35 | ns |
| β-myrcene | 0.09 | 0.15 | p<0.01 |
| β-pinene | 0.02 | 0.07 | p<0.01 |
| limonene 1 | 26.69 | 41.36 | ns |
| limonene 2 | 9.29 | 8.81 | ns |
| **Oxygenated monoterpenes** | **1.59** | **1.15** | **ns** |
| 4-terpineol | 0.23 | 0.19 | ns |
| linalyl alcohol | 1.36 | 0.96 | ns |
| **Sesquiterpenes** | **8.99** | **12.31** | **p<0.01** |
| (-)-aristolene | 0.14 | 0.19 | p<0.05 |
| β-eudesmol | 0.52 | 0.46 | ns |
| muurolene | 1.44 | 1.40 | ns |
| calarene | 3.26 | 0.32 | p<0.05 |
| β-cubebene | 0.46 | 6.66 | p<0.01 |
| (.+-.)-cadinene | 0.30 | 0.19 | ns |
| (-)-β-elemene | 0.12 | 0.36 | ns |
| δ-elemene | 0.35 | 0.48 | ns |
| elemol | 0.17 | 0.14 | ns |
| elixene | 0.31 | 1.96 | ns |
| eudesmene | 1.95 | 0.15 | ns |

*: Area percent represents the mean of ten replicates (t=trace <0.01 %).
F-test: ns stands for no significance; p<0.05 indicates significantly different at 95 % level, while p<0.01 indicates significantly difference at 99 % level.

Lour), each can be considered as a different ecotype. The basic aroma components are the same, but the richness or abundance of certain components varies, particularly in the quantity of esters, which likely causes the difference in sensational evaluation.

NISPEROS-CARRIEDO et al. (1992) used a headspace analysis technique to analyse the volatile aroma of calamondin cultivated in Florida USA. They identified 20 compounds including 5 aldehydes, 2 esters, 5 alcohols, and 8 hydrocarbons. In this study, we extracted the aroma constituents directly from the juice using a SPME method. More compounds were identified than with the headspace technique. This can be attributed to the fact that the absorption fibre is allowed to soak in the juice sample, and thus absorbs both the volatile and non-volatile fragrance components. In addition, the organic components and water-soluble constituents of the juice can also be determined by this method. The SPME method is more valuable for measuring the aroma constituents in citrus juices. Since the method does not require expensive instrumentation setup as the headspace technique, it has great potential for analysing the quality of citrus juice.

SPME fiber coated with poly(dimethylsiloxane) has been used to analyse the flavour components of fresh strawberry juice with no satisfactory results due to the interference of sugar and other nonvolatile components (SCHEPPERS WERCINSKI 1999). Therefore, headspace sampling instead of directly immersing fibre in the sample has been suggested for sampling the fruit juice sample. In this study we identified two broad peaks at retention times 4 and 11 minutes, respectively. They are limonene and methylmaleic anhydride. However, the sampling and analysing system used in this

study has been used to study short chain fatty acid for water sample (YO 1999). The system performed very well for detecting organic acids with carbon number less than 12. We had examined the chromatogram carefully and failed to identify any organic acids in this study. In addition, the sample matrices we are studying contain very low sugar. Therefore the juice matrix interference of the analysis was not likely to happen in this study.

RUBERTO et al. (1997) applied a simultaneous steam distillation technique to collect the essential oils from the fruit of *Citrus clementina x Citrus sinensis*. They identified 42 chemical constituents from the essential oil. Although this species is different from the calamondin, the identified chemical compounds are similar. The SPME method used in this study may be used to replace the tedious simultaneous steam distillation technique in similar studies.

## Acknowledgements

The authors thank Mr. R. M. Yang of TARI, Chiayi for his assistance of fruit photographic job as shown in the title page.

## References

LI, H. L., T. S. LIU, T. C. HUNG, T. KOYAMA and C. E. DE VOL 1979: Flora of Taiwan Vol. 6. Epoch Co., Taipei, Taiwan, 81–82.

LIAO, G.-I., H.-T. HSU, C.-C. WU, C.-S. KUOH, C.-L. LEE and C.-H. LIN 1999: The small citrus fruits in Taiwan. J. Chinese Soc. Hort. Sci. **45**, 37–41.

MORTON, J. E 1987: Calamondin fruits of warm climates. Morton Publisher: Miami, FL, USA, 176–178.

NISPEROS-CARRIEDO, M.O., E.A. BALDWIN, M.G. MOSHNAS and P.E. SHAW 1992: Determination of volatile flavour components, sugars, and ascorbic, dehydroascorbic and other organic acids in Calamodin (*Citrus mitis* Blanco). J. Agric. Food Chem. **40**, 2462–2466.

RUBERTO, G., D. BIONDI, P. RAPISARDA, A. RENDA and A. STARRANTINO 1997: Essential oil of two new pigmented citrus hybrids, *Citrus clementina* x *Citrus sinensis*. J. Agric. Food Chem. **45**, 467–471.

SCHEPPER WERCINSKI, S.A 1999: Solid phase microextraction. Marcel Dekker, Inc. Basel, New York, USA.

YO, S-P 1999: Analysis of volatile fatty acids in wastewater collected from a pig farm by a solid phase microextraction method. Chemosphere **38**, 823–834.

Received November 24, 2002 / Accepted March 31, 2004

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